

**IN THE SPECIFICATION:**

Please replace the paragraph beginning at page 1, line 4, with the following amended paragraph:

This application is a continuation in part (CIP) and claims the benefit of priority under 35 U.S.C. §119 to Japan patent application no. 2000-354396, filed November 21, 2000; and Japan patent application no. ~~20001-190524, filed 6/22/2001~~ 2001-190524, filed June 22, 2001. The aforementioned applications are explicitly incorporated herein by reference in its entirety and for all purposes.

Please replace the paragraph beginning at page 29, line 30, with the following amended paragraph:

The expression vector YEp352GAP-II (PIR1-HA-gma12) was transformed to a yeast strain W303-1A (ura3, leu2, his3, trp1, ade2) (Kainuma et al., Glycobiology, ~~9, 133-141(1999)~~ 9, 133-141(1999)), thereby obtaining a strain W303- YEp352GAP-II(PIR1-HA-gma12).

Please replace the paragraph beginning at page 30, line 9, with the following amended paragraph:

First, the above transformant strain W303-YEp352GAP-II(PIR1-HA-gma12) and a control strain W303-YEp352GAP-II, which had been transformed from a strain W303-1A using YEp352GAP-II, were cultured in 5ml of a SD (-uracil) liquid medium up to OD600=5 (for approximately 30 hours). Then, 1ml of the culture solution was collected, and then the cells were washed with PBS; (8mg / ml NaCl, 0.2mg / ml KCl, 1.44mg / ml Na<sub>2</sub>HPO<sub>4</sub>, 0.24mg / ml ~~KH<sub>2</sub>PO<sub>4</sub>~~ KH<sub>2</sub>PO<sub>4</sub> (pH 7.4)). The cells were collected, suspended in ~~250ml~~ 250μl of a PBS solution ~~□~~ [8mg / ml NaCl, 0.2mg / ml KCl, 1.44mg / ml Na<sub>2</sub>HPO<sub>4</sub>, 0.24 mg / ml KH<sub>2</sub>PO<sub>4</sub> (pH7.4), 1mg / ml ~~BSA~~ BSA] containing ~~4mg~~ 1μg of HA antibody [Anti-HA High Affinity (Roche)], and then incubated on ice for 30 min. The cells were collected and washed once with a PBS solution. Subsequently, the cells were suspended in ~~250ml~~ 250μl of a PBS solution (8mg /

ml NaCl, 0.2mg / ml KCl, 1.44mg / ml ~~Na<sub>2</sub>HPO<sub>4</sub>~~ Na<sub>2</sub>HPO<sub>4</sub>, 0.24mg / ml ~~KH<sub>2</sub>PO<sub>4</sub>~~ KH<sub>2</sub>PO<sub>4</sub> (pH 7.4), 1mg / ml BSA) containing ~~1mg~~ 1μg of a fluorescein secondary antibody (ALEXA FLUOR™ 546 goat anti-rat IgG (H+L) conjugate (Molecular Probe)), and then incubated on ice for 30 min while shielding from light. During their respective incubation for 30 min, the cells and the antibody solution were occasionally mixed by a turning-over method for thorough mixing. The cells were collected, washed twice with PBS, suspended in ~~40ml~~ 40μl of PBS, and then observed with a fluorescence microscope (Fig. 2).

Please replace the paragraph beginning at page 30, line 29, with the following amended paragraph:

Galactosyltransferase activity was measured by referring to Yoko-O et al's method (Yoko-O, Eur. J. Biochem., 257, 630-637 (1998)). As an enzyme source, the yeast intact cell itself (W303-YEp352GAP-II (PIR1-HA-gma12)) prepared in Example 3 was used. As an acceptor substrate, PA-mannobiose was used; as a donor substrate, UDP-galactose was used. A reaction solution of ~~50ml~~ 50μl (100mM HEPES (pH7.2), 1mM MnCl<sub>2</sub>, 5mM UDP-galactose, 300pmol PA-mannobiose) was prepared to contain ~~11ml~~ 11μl of a cell suspension, followed by incubation at 37°C for 5 hours. The cell suspension used herein was prepared by collecting 1ml of a culture solution with OD<sub>600</sub>=6, washing twice with Wash Buffer (10mM Tris-HCl (pH 8), 1mM PMSF), and suspending in ~~11ml~~ 11μl of Wash Buffer (10mM Tris-HCl (pH 8), 1mM PMSF). Then, ~~30ml~~ 30μl of ice-cooled water was added to the reaction solution. The precipitated cells were removed by centrifugation at 3,000 rpm for 3 min, the supernatant with a molecular weight of 10,000 or more was removed with an Ultra Free (~~0.22mm~~ 0.22μm), and then mannobiose and galactosylmannobiose were measured with HPLC. An AMIDE-80™ column (TSK gel AMIDE-80™, TOSOH, 0.46cm in diameter x 25cm in length) was used for HPLC. A mixture A containing 200mM acetic acid-triethylamine buffer (pH 7.0) and acetonitrile (10 : 90), and a mixture B containing 200mM acetic acid-triethylamine buffer (pH 7.0) and acetonitrile (60 : 40) were prepared. The column had been previously equilibrated by running the solvent A through the column at a flow rate of 1.0ml/min. Immediately after injection of

samples, the proportion of the solvent B was raised linearly for 60 min up to 100%, so that PA-oligosaccharide was eluted.

Please replace the paragraph beginning at page 32, line 3, with the following amended paragraph:

A plasmid pBS(SK-)/FT6H1.3 (provided by Dr. Narumatsu of Soka University) containing the amino acid coding region of a FUT6 gene, which is a human  $\alpha$ -1,3-FucT (DB name: GenBank; Accession No: L01698) ~~□ Weston~~ (Weston, J. Biol. Chem., 267, 24575-24585 (1992)), was used as a template. A primer previously containing an SalI site on the N-terminal side and an XhoI site on the C-terminal side was designed to enable amplification except for a transmembrane region located on the N-terminal side of an FUT6 protein. Primers having the following base sequences were used.

Please replace the paragraph beginning at page 33, line 5, with the following amended paragraph:

First, the above transformant strain W303-YEp352GAP-II(PIR1-HA-FUT6) and a control strain W303-YEpGAP-II, which had been transformed from a strain W303-1A using YEp352GAP-II, were cultured in 5ml of an SD (-uracil) liquid medium to OD600=5 (for approximately 30 hours). Then, 1ml of the culture solution was collected, and then the cells were washed with PBS [8mg / ml NaCl, 0.2mg / ml KCl, 1.44mg / ml ~~Na<sub>2</sub>HPO<sub>4</sub>~~ Na<sub>2</sub>HPO<sub>4</sub>, 0.24mg / ml KH<sub>2</sub>PO<sub>4</sub> (pH7.4)]. The cells were collected, suspended in ~~250ml~~ 250μl of a PBS solution (8mg / ml NaCl, 0.2mg / ml KCl, 1.44mg / ml ~~Na<sub>2</sub>HPO<sub>4</sub>~~ Na<sub>2</sub>HPO<sub>4</sub>, 0.24mg / ml ~~KH<sub>2</sub>PO<sub>4</sub>~~ KH<sub>2</sub>PO<sub>4</sub> (pH 7.4), 1mg / ml BSA) containing ~~1mg~~ 1μg of HA antibody [Anti-HA High Affinity (Roche)], and then incubated on ice for 30 min. The cells of each strain were collected respectively and washed once with a PBS solution. Subsequently, the cells were suspended in ~~250ml~~ 250μl of a PBS solution (8 mg / ml NaCl, 0.2mg / ml KCl, 1.44mg / ml ~~KH<sub>2</sub>PO<sub>4</sub>~~ KH<sub>2</sub>PO<sub>4</sub>, 0.24mg / ml ~~KH<sub>2</sub>PO<sub>4</sub>~~ KH<sub>2</sub>PO<sub>4</sub> (pH7.4), 1mg / ml BSA) containing ~~1mg~~ 1μg of a labeled secondary antibody (Alexa FLUOR™ 546 goat anti-rat IgG (H+L) conjugate (Molecular Probe)), and then incubated

on ice for 30 min while shielding from light. During their respective incubation for 30 min, the cells and the antibody solution were mixed occasionally by a turning-over method for thorough mixing. The cells were collected, washed twice with PBS, suspended in ~~40ml~~ 40µl of PBS, and then observed with a fluorescence microscope.

Please replace the paragraph beginning at page 33, line 25, with the following amended paragraph:

Fucosyltransferase activity was measured by referring to GLYCOBIOLOGY Experimental Protocol (ed. Taniguchi et al., 156-159 (1996)). As an enzyme source, a solution was prepared by disrupting yeast cells (W303-YEp352GAP-II(PIR1-HA-FUT6), prepared in Example 6) with glass beads in Wash Buffer (10mM Tris-HCl (pH8), 1mM PMSF). PA-Lacto-N-neotetraose was used as an acceptor substrate; GDP-fucose was used as a donor substrate. 5.5 µl of the cell disruption solution was added to 4.5 µl of a reaction solution (50mM Cacodylate buffer (pH 6.8), 5mM ATP, 25 mM ~~MnCl2~~ MnCl<sub>2</sub>, 0.075 mM GDP-fucose, 0.075 mM PA-Lacto-N-neotetraose), followed by incubation at ~~37~~ 37°C for 5 hours. A cell suspension used herein was a solution containing disrupted cells which had been prepared by collecting 0.25ml of the culture solution with OD<sub>600</sub>=6, washing twice with Wash Buffer (10mM Tris-HCl (pH 8), 1mM PMSF) and then crushing with glass beads. Next, to stop reaction, incubation was performed at 98°C for 3 min, and then ~~40ml~~ 40µl of ice-cooled water was added to the reaction solution. The precipitated cells were removed by centrifugation at 3,000rpm for 3 min, and then the supernatant with a molecular weight of 10,000 or more was removed with an ULTRA FREE™ (~~0.22mm~~ 0.22µm). Subsequently, Lacto-N-neotetraose and Lacto-N-fucopentaose were measured with HPLC. An Amide-80 column (TSK gel Amide-80, TOSOH, 0.46cm in diameter x 25cm in length) was used for HPLC. A mixture A containing 200mM acetic acid-triethylamine buffer (pH 7.0) and acetonitrile (10 : 90), and a mixture B containing 200mM acetic acid-triethylamine buffer (pH 7.0) and acetonitrile (60 : 40) were prepared. The column had been previously equilibrated by running the solvent A through the column at a flow rate of 1.0ml/min.

Immediately after injection of samples, the proportion of the solvent B was raised linearly for 60 min to 100%, so that PA-oligosaccharide was eluted.

Please replace the paragraph beginning at page 37, line 20, with the following amended paragraph:

The expression vectors YEp352GAP-II (PIR1-HA-KRE2) and YEp351GAP-II(PIR2-FLAG-MNN1) were transformed simultaneously to a yeast strain W303-1A (ura3, leu2, his3, trp1, ade2) ~~(Kainuma et al., Glycobiology, 9:133-141(1999))~~ (Kainuma et al., Glycobiology, 9, 133-141(1999)), thereby obtaining a strain W303-YEp352GAP-II(PIR1-HA-KRE2), YEp351GAP-II(PIR2-FLAG-MNN1). The strain W303-YEp352GAP-II(PIR1-HA-KRE2), YEp351GAP-II(PIR2-FLAG-MNN1) was deposited under the Accession No. FERM BP-7789 at the International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology on June 20, 2001.

Please replace the paragraph beginning at page 38, line 1, with the following amended paragraph:

First, the above transformant strain W303-YEp352GAP-II(PIR1-HA-KRE2), YEp351GAP-II(PIR2-FLAG-MNN1) and a control strain W303-YEp352GAP-II, which had been transformed from a strain W303-1A using YEp352GAP-II, were cultured in 5ml each of an SD (-uracil, -leucine) and an SD(-uracil) liquid media to OD600=5 (for approximately 48 hours). Then, the cells were collected and washed with Wash Buffer (10mM Tris-HCl (pH8.0), 1mM PMSF). Glass beads were added to the cell suspension and Vortex was applied at 4°C for 15 min, so that the cells were disrupted. The solution containing the disrupted cells was separated into supernatant (Lane 1 of Fig. 9A and B) and pellet. The pellet was washed three times with Wash Buffer (10mM Tris-HCl (pH8.0), 1mM PMSF), suspended in ~~100ml~~ 100µl of Laemmli Buffer (4% [4% SDS, 20% glycerol, 0.12M Tris-HCl (pH6.6), 8M urea, 2%β-ME] ~~2%β-ME~~), and then boiled at ~~100~~ 100°C for 10 min. This sample was centrifuged and further separated into supernatant (Lane 2 of Fig. 9A and B) and pellet fraction. The pellet fraction was washed

three times with Na-acetate Buffer (pH 5.5) [0.1M Na-acetate], and then incubated with ~~100ml~~ 100μl of a mild alkali solution [30mM NaOH] at 4°C for 15 hours. The suspension with the mild alkali solution was centrifuged, so that supernatant was collected (Lane 3 of Fig. 9A and B). The collected samples were subjected to SDS-PAGE, and then Western blotting. At this time, primary antibodies used herein were HA antibody ~~MONOCLONAL ANTIBODY, HA.11 (CONVANCE)~~ (MONOCLONAL ANTIBODY, HA.11 (CONVANCE)) and FLAG antibody-ANTI-FLAG M2 Monoclonal Antibody (SIGMA); and a secondary antibody used herein was anti-mouse IgG-HRP-Anti-Mouse IgG (H&L) HRP-Linked Antibody ( Cell Signaling TECHNOLOGY). To perform immuno-staining with 2 types of antibodies, HA antibody and FLAG antibody, two membranes to which the same protein solution had been blotted were prepared, and then immuno-staining was performed separately with the 2 types of antibodies. Thus, a specific band was detected only for a strain expressing the fusion protein when the cell wall fraction was treated with mild alkali. This result reveals that Pir1-HA-Kre2 fusion protein and Pir2-FLAG-Mnn1 fusion protein were localized simultaneously on a cell wall with a binding pattern (to a cell wall) representing the characteristics of PIR.

Please replace the paragraph beginning at page 38, line 29, with the following amended paragraph:

Mannosyltransferase activity was measured by referring to Lussier et al's method (Lussier et al., JBC., 271, 11001-11008 (1996)). As an enzyme source, the yeast intact cell itself (W303- YEp352GAP-II(PIR1-HA-KRE2), YEp351GAP-II(PIR2-FLAG-MNN1)) prepared in Example 12 was used. A control strain used herein was W303-YEp352GAP-II. As an acceptor substrate, PA-mannobiose was used; as a donor substrate, GDP-mannose was used. A reaction solution ~~50ml~~ 50μl (100mM HEPES(pH7.2), 1mM ~~MnCl2~~ MnCl<sub>2</sub>, 5mM GDP-mannose, 300pmol PA-mannobiose) was prepared to contain ~~20ml~~ 20μl of a cell suspension, followed by incubation at 37 °C for 3 hours. The cell suspension used herein was prepared by collecting 1ml of a culture solution with OD600=4, washing twice with Wash Buffer (10mM Tris-HCl(pH8), 1mM PMSF), and then suspending in ~~20ml~~ 20μl of Wash Buffer. Then, ~~50ml~~ 50μl of ice-cooled

water was added to the reaction solution. The precipitated cells were removed by centrifugation at 3,000rpm for 3 min, and then the supernatant with a molecular weight of 10,000 or more was removed with an Ultra Free (~~0.22mm~~ 0.22µm). Then, mannobiose (disaccharide), mannotriose (trisaccharide) and mannotetraose (tetraose) were detected with HPLC. Amide-80 column (TSK gel An Amide-80, TOSOH, 0.46cm in diameter x 25cm in length) was used for HPLC. A mixture A containing 200mM acetic acid-triethylamine buffer (pH 7.0) and acetonitrile (10 : 90), and a mixture B containing 200mM acetic acid-triethylamine buffer (pH 7.0) and acetonitrile (60 : 40) were prepared. The column had been previously equilibrated by running the solvent A through the column at a flow rate of 1.0ml/min. Immediately after injection of samples, the proportion of the solvent B was raised linearly for 60 min to 100%, so that PA-oligosaccharide was eluted.